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Synthesis of 7β-[(*Z*)-2-(2-Amino-4-thiazolyl)-2-methoxyiminoacetamido]-3-cephem-4-carboxylic Acid (Ceftizoxime), a New Semisynthetic Cephalosporin Antibiotic. I. An Improved Method for the Preparation of 7-Amino-3-methylene- and 7-Amino-3-hydroxycepham-4-carboxylic Acid

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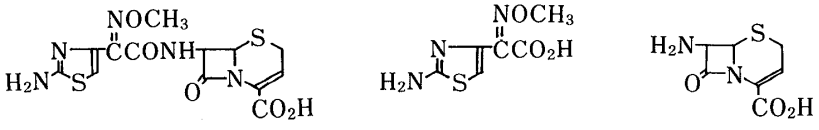
New synthetic methods for 7-amino-3-methylenecepham-4-carboxylic acid (X) and 7-amino-3-hydroxycepham-4-carboxylic acid (IX), which are key intermediates for the synthesis of ceftizoxime (I), were developed. These intermediates (IX and X) were synthesized without introducing any protecting groups at the C-7 amino and/or C-4 carboxylic acid groups of the cepham or cephem ring. Compound X was prepared from 7-amino-3-(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl-3-cephem-4-carboxylic acid (XI) by zinc reduction either in aqueous acidic or anhydrous neutral conditions. Compound IX was prepared from X by ozone oxidation at −75 °C followed by sodium borohydride reduction at 0—10 °C. A plausible mechanism of ozone oxidation of X is presented, and the existence of an important intermediate, 7-amino-3-hydroxy-3-cephem-4-carboxylic acid (XXII), is demonstrated.

Keywords——cefrizoxime; synthesis; 7-amino-3-methylenecepham-4-carboxylic acid; ozone dergradation; 7-amino-3-hydroxycepham-4-carboxylic acid; stereostructure; 7-amino-3-hydroxy-3-cephem-4-carboxylic acid

In the course of a project on the synthesis of ceftizoxime (CZX) (I),[2)] we have been engaged in the development of a procedure for the manufacture of I. One of our synthetic strategies was to avoid, as much as possible, the problems associated with introduction and removal of protecting groups of the amino group at C-7 and/or the carboxyl group at C-4 of the cepham or cephem ring.

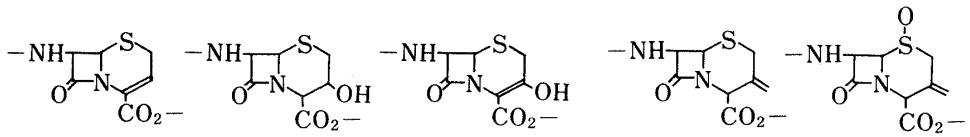
I is comprised of (*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino)acetic acid (II) and 7-amino-3-cephem-4-carboxylic acid (III) moieties. The synthesis of 3-unsubstituted-3-cephem rings (IV) has been reported by several groups.[3a−d)] First, Heusler and Fechtig synthesized IV from a penicillin derivative.[3b)] However, the preferred routes to the 3-unsubstituted derivative (IV) are either *via* the 3-exomethylene-cepham derivative (VII)[4a−l)] or the 3-hydroxy-3-cephem derivative (VI).[5a−e)]

The synthesis of 3-exomethylenecepham compounds (VII) has been achieved only under limited conditions because of the labile β-lactam structure of the molecule. So far, VII has been synthesized from 3-substitutedthiomethyl-3-cephem compounds by reduction with Raney nickel[4a,b)] or Zn–HCO_2H–dimethylformamide(DMF),[4b)] and from 3-acetoxymethyl-3-



ceftizoxime I II III

Fig. 1



IV V VI VII VIII

Fig. 2

cephem compounds by reduction with Cr (II),[4c] Hg (Al)[5a] or electrolysis.[4d] Compound VII was also synthesized by ring expansion of penicillin sulfoxide.[4c-l]

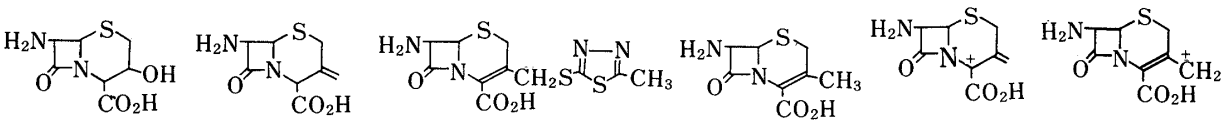
Our synthetic plan was to lead to III *via* 7-amino-3-hydroxycepham-4-carboxylic acid (IX) for the synthesis of I. We intended to prepare IX from 7-amino-3-methylenecepham-4-carboxylic acid (X) *via* ozonolysis followed by reduction of 7-amino-3-hydroxy-3-cephem-4-carboxylic acid (XXII) without introduction of any protecting groups.

7-Amino-3-(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl-3-cephem-4-carboxylic acid (XI) was chosen as a starting material for the reduction of 3-substituted thiomethyl-3-cephem-4-carboxylic acid to the 3-exomethylene compound (X), because XI could be obtained in a good yield from cephalosporin C and is an intermediate for cefazolin (CEZ) which is a widely used cephalosporin antibiotic. Although Chauvette and Pennington have reported the synthesis of X from 3-substitutedthiomethyl-3-cephem-4-carboxylic acid by Zn–HCO_2H–DMF reduction, they emphasized the importance of coexistence of HCO_2H and DMF.[4b] We found that there is no need for coexistence of HCO_2H–DMF, and X was produced from XI in a good yield with aqueous HCl and Zn. At the same time, we found that XI was reduced to the 3-exomethylene compound (X) under anhydrous neutral conditions in better yield than under aqueous acidic conditions. Although the 3-chloromethyl-3-cephem compound wal also reported to be reduced to the 3-exomethylene compound (X) under anhydrous conditions,[4j,k] this is the first report of converting a 3-substituted thiomethyl compound such as XI into the 3-exomethylene compound (X) under anhydrous neutral conditions. A 3-substituted thiomethyl compound such as XI has another advantage of not cyclizing into the inactive lactone as the 3-chloromethyl-3-cephem compound does.

Preparation of X from XI: The Zn reduction of XI to X and XII was examined in various reaction mixtures containing HCl, H_2SO_4 and *p*-toluenesulfonic acid (*p*-TsOH), and the results are shown in Table I. Compound X was produced in good yields when reduction was carried out using H_2O as a solvent, but addition of an organic solvent, protic or aprotic, in order to suppress the isomerization to XIV of the initially formed intermediate (XIII) produced by the proposed push-pull mechanism[4l] only resulted in an increase of the undesired compound (XII). On the other hand, when the temperature of the reaction mixture was lowered to -15 °C to stabilize the initially formed intermediate (XIII), the highest yield of 78.1% of X was achieved. Compound X was precipitated from the reaction mixture at its isoelectric point (pH 3.6).

Compound X was also found to be produced preferentially even under anhydrous conditions with Zn in the presence of ammonium chloride (NH_4Cl) in dry DMF, although the addition of an organic solvent to the aqueous HCl–Zn reduction stated above lowered the yield of X. Compound XI was treated with bis (trimethylsilyl) urea (BSU) in dry DMF, and then allowed to react with Zn in the presence of NH_4Cl at 5 °C with vigorous stirring to give a mixture of X and XII in a product ratio of 27 : 1 (Table II, run 3). When the stirring was continued after the starting XI was exhausted, the yield of X was found to diminish with a concomitant increase of XII (Table II, runs 3 and 4). Thus, the double bond isomerization of X to XII was facilitated by ammonia produced by the reaction of NH_4Cl and Zn, and this isomerization was accelerated with a rise in temperature (Table II, run 5).

As was shown in Tables I and II and discussed above, the product ratio of X : XII is much higher under anhydrous neutral conditions than under aqueous acidic conditions. Since



IX X XI XII XIII XIV

Fig. 3

TABLE I. Preparation of X

Solvent	Acid	Reaction			Yield (%)		
		Mole ratio	Temp. (°C)	Time (min)	X	XII	Unchanged XI
H_2O	*p*-TsOH	10	20	75	73.5	13.8	0
H_2O	H_2SO_4	9	0	180	73.9	20.7	0
H_2O	HCl	6	5	160	75.2	12.4	0
$H_2O/CaCl_2$	HCl	9	−15	180	78.1	15.4	0
50% MeOH	*p*-TsOH	10	20	20	45.6	40.5	0
50% DMSO	*p*-TsOH	10	0	30	67.9	21.0	0
50% DMA	*p*-TsOH	10	0	60	46.6	42.1	1
50% AcOH	HCl	9	0	300	44.3	35.2	0

Yields of X, XI and XII were calculated from HPLC data using analytically pure samples as standards (see Experimental for HPLC conditions).

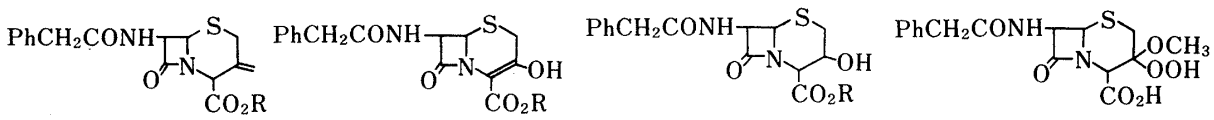
TABLE II. Preparation of X under Anhydrous Conditions[a)]

Run	Reaction temp. (°C)	Reaction time (h)	Additional stirring time (h)	Yield (%)[b)]	
				X	XII
1	−10	2	0	0	0
2	0	2	0	81.3	5.0
3	5	1.5	0	83.4	3.0
4	5	1.5	1.3	79.9	5.0
5	12	0.8	0	74.4	10.5

a) Other experimental conditions except those shown in Table II were the same as those for the preparation of X from XI (see procedure B in Experimental). *b*) Yields were calculated from HPLC data using an analytical sample as a standard.

double bond migration from the 3-exomethylenecepham compound (X) to the 3-methyl-3-cephem compound (XII) cannot occur under acidic conditions, the difference between the product ratios under the two conditions can be ascribed to different reaction mechanisms. The reaction under aqueous acidic conditions is generally said to proceed through an ionic mechanism, while that under anhydrous neutral conditions proceeds through a concerted mechanism.

Preparation of IX: The ozonolysis of the exomethylene function of cephalosporins (X or XV) needs special attention as regards 1) suppression of sulfoxide formation,[5b)] 2) selective oxidation of the exomethylene function and 3) suppression of decarboxylation from the formed β-ketocarboxylic acid.[5a)] First, we tried some preliminary ozonolysis experiments using XV on the basis of the results reported by Scartazzini and Bickel.[5a)] When XV was allowed to react with ozone in CH_2Cl_2 at −65 °C and treated in the same way, the desired product (XVII) was not produced. On the other hand, ozonolysis of XV in MeOH at −65 °C gave a positive ferric chloride test, an indication of enolic function. The reaction mixture obtained was treated with excess diphenyldiazomethane at −65 °C to give the keto-ester (XVIII), which was identified by comparison of its spectral data with those of an authentic



XV: R=H
XVI: R=$CHPh_2$
XVII: R=H
XVIII: R=$CHPh_2$
XIX: R=H
XX: R=$CHPh_2$
XXI

Fig. 4

sample prepared by the method described in the literature.[5a] When the isolated keto-ester (XVIII) was treated with $NaBH_4$ at −65 °C in MeOH, the ferric chloride test became negative, giving rise to XX in a good yield. The structure of XX was confirmed by comparing its spectral data with those of an authentic sample.[5a] Next, direct reduction of the ozonolysis intermediate starting from XV was examined. When the ozonolysis reaction mixture from XV was treated with $NaBH_4$ at −65 °C, the ferric chloride test was still positive, and this indicated that the reducing reaction to XIX was not proceeding under these conditions, contrary to the case of XVIII. But, when the ozonolysis reaction mixture from XV was treated with $NaBH_4$ in an aqueous alkaline solution in the temperature range of 0 to 10 °C, the 3-hydroxycepham compound (XIX) was produced in 71.0% yield. From these findings, we assumed that an initial intermediate of this ozonolysis reaction at −65 °C was the perhydroxyketal (XXI),[6a,b] and that such an intermediate might be stable in MeOH and resistant to $NaBH_4$ reduction at −65 °C but be converted at a high temperature into a β-keto-derivative (XVII), which was easily reduced with $NaBH_4$ to XIX before decarboxylation.[5a]

On the basis of these findings, we started to study ozonolysis of X. The methanesulfonate or hydrochloride of X was treated with ozone at −75 °C in dry MeOH. The reaction mixture showed a positive ferric chloride test at room temperature. The ozonolysis mixture was worked up in two ways: (i) isolation of semi-stable intermediate, and (ii) reduction with $NaBH_4$.

Isolation of semi-stable intermediate: To simplify the assignment of the intermediate by proton magnetic resonance (^{1}H-NMR) spectroscopy, the hydrochloride of X was used as the starting material. Careful removal of MeOH and volatile materials after ozonolysis gave rise to a crystalline product which was positive in the ferric chloride test in aqueous MeOH. The infrared (IR) and nuclear magnetic resonance (NMR) spectra of the product showed the presence of a cephalosporin nucleus. When the product was treated with $NaBH_4$ in aqueous MeOH, IX was produced in 39.9% yield. We therefore assumed the isolated material to be XXII, which possesses appreciable stability even at room temperature in spite of having a β-keto-carboxylic acid moiety, contrary to the report by Scartazzini and Bickel.[5a]

Reductive treatment: Ozonolysis of the methanesulfonate of X was carried out at various temperatures, and the resulting ozonolysis mixture was treated immediately with $NaBH_4$ at 0—10 °C in all experiments. The results are shown in Table III. The yield of IX was found to increase with a decrease in the ozonolysis temperature. When the ozonolysis mixture was kept at −50 °C for 1 h following ozonolysis at −75 °C, IX was produced in the highest yield of 83.0%, whereas the yield was 75% when the ozonolysis mixture was reduced immediately. This again supports the idea of an initial formation of a perhydroxyketal intermediate (Fig. 10), as was discussed under the ozonolysis of XV. When ozone passage was conducted at a higher temperature (−40 °C), the main product was found to be the 1-sulfoxide (XXIII), which was isolated as a phenylacetyl derivative (XXIV). This suggests that the sulfoxide function provides chemical resistance to the exomethylene group against ozone attack, by its steric hindrance. Compound IX produced was precipitated from the reaction mixture at its isoelectric point (pH 4.0) in a good yield.

Absolute structure of IX: The 270 MHz NMR spectrum is shown in Fig. 6. Analysis of

TABLE III. Preparation of IX from X[a]

Run	Reaction temp. with O_3 (°C)	After-treated	Yield (%) IX[b]	Yield (%) XXIII[c]
1	−40	None	13.9	22
2	−65	None	40.0	ND[d]
3	−75	None	75.0	ND
4	−75	−50 °C for 1 h	83.0	ND

a) Other experimental conditions except those shown in Table III were the same as for the preparation of IX from X (see Experimental). *b*) Yield in the solution. Yield of IX was calculated from HPLC data using an analytically pure sample as a standard (see Experimental for HPLC conditions). *c*) Compound XXIII was isolated as the phenylacetyl derivative (XXIV). *d*) ND: Not detected.

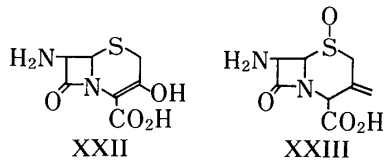


Fig. 5

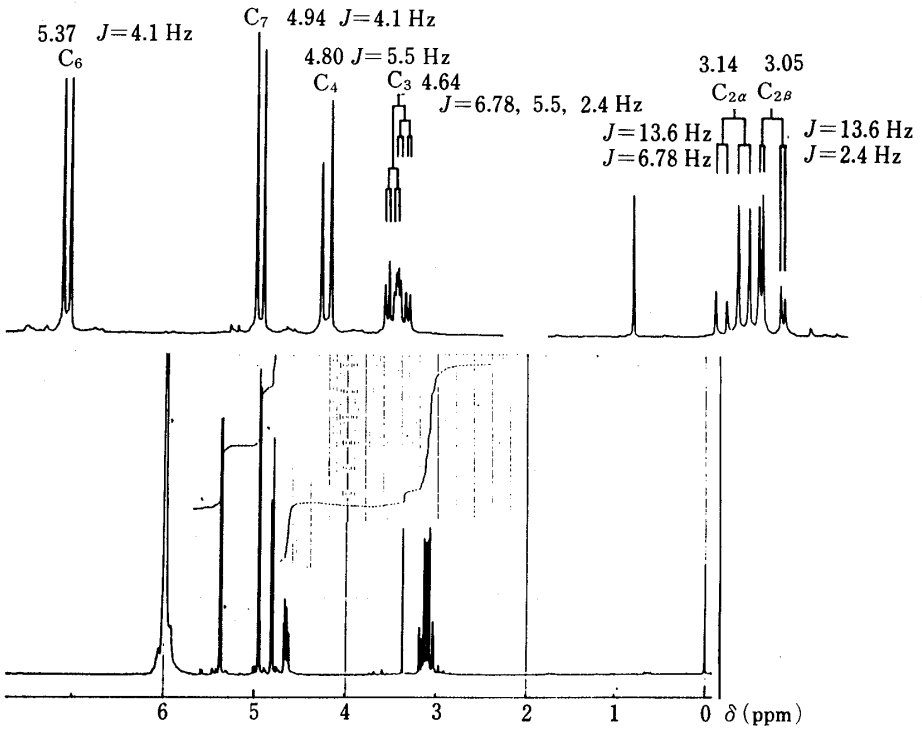


Fig. 6. Spectral Data for IX

A, 270 MHz ^{1}H-NMR spectrum; B, expanded spectrum of A and its complete assignment.

chemical shifts, coupling constants and nuclear Overhauser effect (NOE) data (Fig. 7) enabled us to assign all the protons shown in Fig. 6. Observed NOEs between $C_{2\beta}$-H and C_4-H, and C_4-H and C_3-H suggest that these three protons are aligned on the same side of the dihydrothiazine ring of the cepham nucleus (Fig. 7). Consequently, the hydroxyl group at C-3 and the carboxyl group at C-4 could be assigned α configurations. The 400MHz two dimensional NOE (2D-NOE) and the NOE difference spectra support this configuration (Figs. 8 and 9, respectively). The same configuration was also reported by Aoki *et al.*[7] for 7α-acylamido-1-oxa-3-hydroxycepham-4-carboxylate. Thus, sodium borohydride reduction of

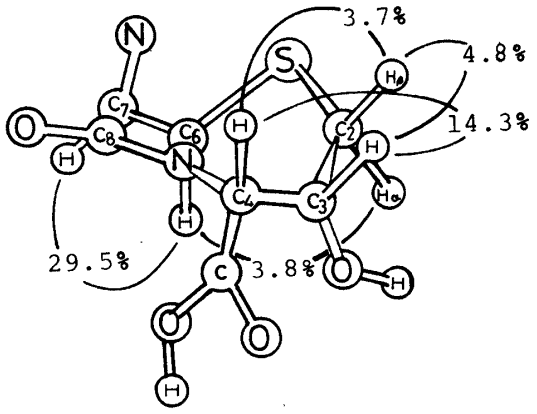


Fig. 7. Assumed Conformation of IX with NOE Data

NOE between C_6 and $C_{2\alpha}$ = 3.8%, $C_{2\beta}$ and $C_{3\beta}$ = 4.8%, $C_{2\beta}$ and $C_{4\beta}$ = 3.7%, $C_{3\beta}$ and $C_{4\beta}$ = 14.3%, and C_6 and C_7 = 29.5%.

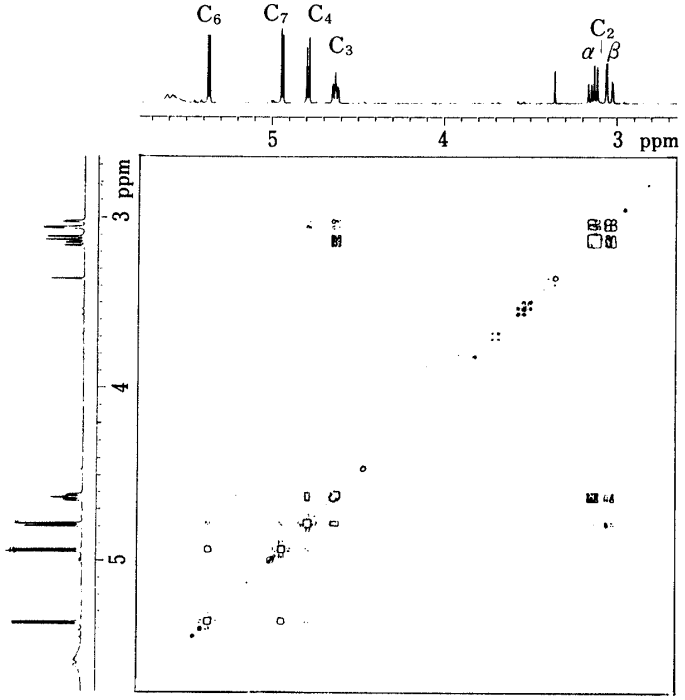


Fig. 8. 2D-400 MHz NOESY Spectrum of IX

7β-amino-3-hydroxy-3-cephem-4-carboxylic acid (XXII) occurred stereoselectively from the upper face of the cephem nucleus, as was the case with 7α-acylamido-1-oxa-3-hydroxy-3-cephem-4-carboxylate, although the configuration at C-7 was opposite. The spin–spin coupling constants, $J_{2\beta,3}$ = 2.4 Hz and $J_{2\alpha,3}$ = 6.78 Hz, agree with the data on XX produced from XVIII by $NaBH_4$ reduction,[5a] and suggest that the relation of $C_{3\beta}$–$C_{2\alpha}$ configuration is not *trans* diaxial, but rather that the C_3 proton is bisected by the $C_{2\alpha}$ and $C_{2\beta}$ protons. The absolute structures of several cephalosporins[8a] and I[8b] have already been determined by X-ray structure analysis. Consequently, the absolute structure of IX is assumed to be as shown in Fig. 7.

On the basis of published information,[6a,b] a plausible reaction pathway for the formation of XXII is illustrated in Fig. 10. The acid salt of X reacts with ozone at −75 °C to afford a Criegee zwitterion (B). Recombination of B with the formaldehyde produced may give an ozonide (E) in a non-polar solvent. However, ozonolysis in MeOH gives C by the addition of MeOH to B. The hydroperoxide (C) is converted into XXII *via* the hemi-ketal (D) when the temperature is raised to −50 °C. It is very interesting that XXII exists in a β-keto-carboxylic form in a slightly acidic medium without any degradation to undesirable materials.

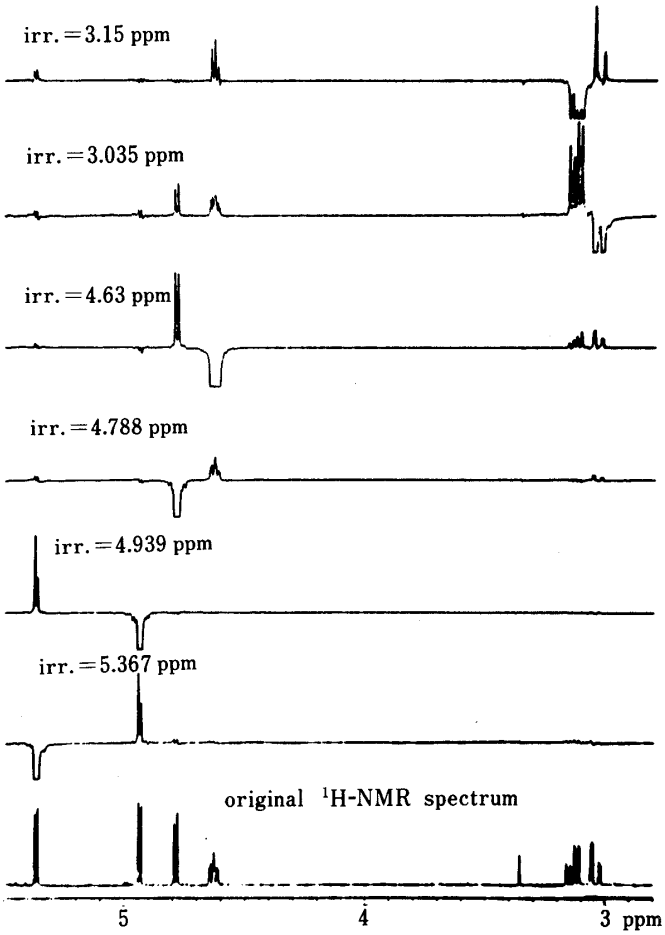


Fig. 9. Difference NOE Spectrum Obtained from 2D-400 MHz NOESY Spectrum (Fig. 8) When Designated Field Was Irradiated

The original ^{1}H-NMR spectrum is shown in the lowest chart.

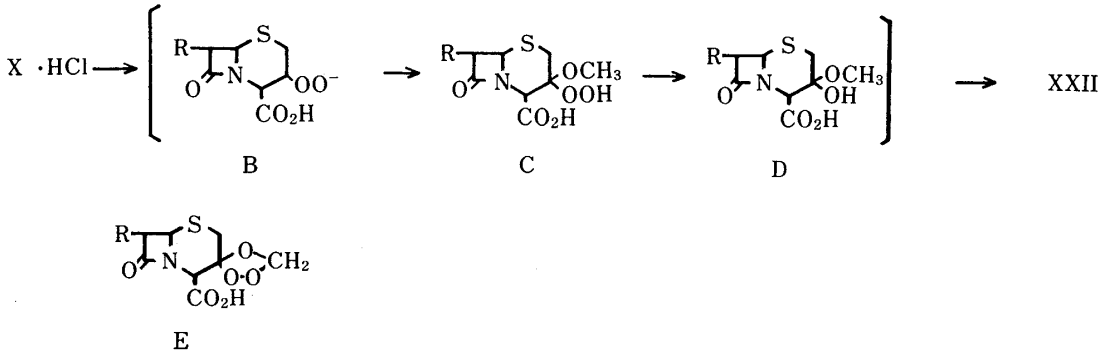


Fig. 10. Reaction Pathway of O_3 Degradation of X into XXII

Experimental

Melting points were measured on a Thomas Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded with Hitachi grating IR spectrophotometer, model 215. ^{1}H-NMR spectra were recorded with a JEOL JNK-PMX 60 NMR spectrometer (60 MHz) or a JEOL MH-100 spectrometer (100 MHz). Chemical shifts are given in (ppm) values using tetramethylsilane in DMSO-d_6, trimethylsilylpropionic acid sodium salt in D_2O–$NaHCO_3$ or trimethylsilylpropanesulfonic acid sodium salt in D_2O–DCl as an internal standard. NOE, nuclear

Overhauser effect spectroscopy (NOESY) and NOE difference spectrum were recorded with JNM-FX 270 and JNM-GX 400 spectrometers in 10% DCl–D_2O after degassing of the sample solution. High performance liquid chromatography (HPLC) was performed on a Hitachi 635A instrument with a multiwavelength ultraviolet (UV) monitor; column, μ-Bondapack-NH_2 3.9 × 300 mm; mobile phase, acetic acid (5 ml), acetonitrile (100 ml), MeOH (100 ml) and H_2O (800 ml); flow rate, 1.5 ml/min; detector, UV_{230} nm. Kiesel gel (0.05—0.2 mm) was used for column chromatography. Microelemental analysis (C,H,N,S) were performed at the Analytical Research Laboratories of Fujisawa Pharmaceutical Co., Ltd.

Commercially available reagents were used without further purification. Anhydrous solvents were dried over Molecular Sieves 3A before use. Diphenyldiazomethane was prepared from benzophenone hydrazone according to the method described in the literature.[9] XI was produced by Fujisawa Pharmaceutical Co., Ltd. Authentic samples of the following compounds have been reported in the literature: X,[4b,d] 7-amino-3-methyl-3-cephem-4-carboxylic acid (XII),[10] 3-methylene-7-phenylacetamidocepham-4-carboxylic acid (XVI)[4c,5a] and its benzhydrylester (XVI),[5a] 3-hydroxy-7-phenylacetamido-3-cephem-4-carboxylic acid benzhydrylester (XVIII),[5a] 3-hydroxy-7-phenylacetamido-cepham-4-carboxylic benzhydrylester (XX).[5a]

7-Amino-3-methylenecepham-4-carboxylic Acid (X)——Procedure A: Concentrated HCl (66 ml) was added dropwise to a suspension of XI (34.5 g; purity = 87.5%), zinc dust (26.4 g) and $CaCl_2$ (17.6 g) in H_2O (240 ml) over 20 min with vigorous stirring, the temperature being kept below 5 °C. The resulting mixture was stirred for 2 h at −10 °C (X in the solution was found to be produced in 78.1% yield based on HPLC analysis), then zinc and other insoluble materials were filtered off and washed with chilled H_2O (25 ml). The filtrate and washing were combined, diluted with MeOH (300 ml), adjusted to pH 3.6 with 48% NaOH aqueous solution and stirred for 2 h at 0—5 °C. The precipitates obtained were collected, washed with H_2O, MeOH and acetone, successively, and dried under reduced pressure to give crude X (13.4 g). The crude powder was dissolved in 6 N HCl (15 ml) and recrystallized by adjusting the pH to 3.6 with 48% NaOH aqueous solution at 5 °C to give X as an analytically pure product in 47.4% yield, mp 213—221 °C (dec.) (lit. 212—214 °C (dec.)).[4d] *Anal.* Calcd for $C_8H_{10}N_2O_3S$: C, 44.85; H, 4.71; N, 13.08; s, 14.97. Found: C, 44.70; H, 4.64; N, 13.02; S, 14.71. IR ν_{max}^{Nujol} cm^{-1}: 3200—2200, 1780, 1620, 1540. NMR (D_2O–$NaHCO_3$, 100 MHz) δ:3.27, 3.83 (2H, AB q, J = 14 Hz, C_2), 5.00, 5.28 (2H, each singlet, $C_3=CH_2$), 5.33 (1H, d, J = 3 Hz, C_4), 5.42 (1H, d, J = 3 Hz, C_7). C_6 proton overlaps with HDO signal. Retention times in HPLC: X, 4.5 min; XII, 5.0 min; XI, 9.0 min.

Procedure B: A mixture of XI (34.5 g; purity = 87.5%) and BSU (35.0 g) in dry DMF (70 ml) was stirred at 25 °C for 13 min, and at 5 °C for 18 min. NH_4Cl (16.1 g) and zinc dust (39.4 g) were added to the resulting clear solution and the mixture was stirred for 30 min at 5 °C. Ice (7 g) and concentrated HCl (0.6 ml) were added, and then the mixture was filtered to remove insoluble materials. The residue on the filter was washed with DMF (15 ml). The filtrate and washings were combined and X was determined by HPLC (83.4% yield). The solution obtained was adjusted to pH 3.6 with concentrated HCl, the temperature being kept below 10 °C. The precipitated product was collected, washed with chilled H_2O, MeOH and acetone, successively, and dried under reduced pressure to give crude X (23.5 g). The powder was recrystallized as under procedure A to afford analytically pure X in 66.5% yield.

3-Hydroxy-7-phenylacetamido-3-cephem-4-carboxylic Acid Benzhydrylester (XVIII) from XV——O_3 gas was passed into a solution of XV (600 mg) in dry MeOH (30 ml) for 5 min at −65 °C. N_2 gas was bubbled into the solution for 5 min to remove excess O_3. After addition of dimethylsulfide (0.2 ml), the solution was stirred for 30 min at −60 °C, and then diphenyldiazomethane was added. The mixture was warmed to room temperature, and stirred for 1 h. H_2O (10 ml) and AcOEt (50 ml) were added, and the aqueous and AcOEt layers were separated. The AcOEt layer was washed with 2 N HCl and brine, successively, and dried over $MgSO_4$. The AcOEt solution was concentrated under reduced pressure to give an oil which was subjected to chromatography over SiO_2 (12 g) using $CHCl_3$ as an eluent to afford XVIII (0.22 g). The compound obtained was identified as XVIII by comparing its spectral data with those of an authentic sample prepared by the method of Scartazzini and Bickel.[5a]

3-Hydroxy-7-phenylacetamidocepham-4-carboxylic Acid (XIX)——O_3 gas was passed into a solution of XV (600 mg) in dry MeOH (30 ml) for 5 min at −65 °C, and N_2 gas was bubbled into the solution for 5 min to remove excess O_3. The ozonized solution was poured into a $NaBH_4$ solution (prepared from $NaBH_4$ (35 mg) and H_2O (10 ml)), the temperature being kept below 10 °C. The mixture was saturated with NaCl, and washed with AcOEt, then the aqueous layer was acidified with 6 N HCl and extracted with AcOEt (60 ml × 3). The extract was washed with H_2O, dried over $MgSO_4$, and concentrated under reduced pressure to give XIX as an oil (430 mg). IR ν_{max}^{Nujol} cm^{-1}: 3300, 3050, 1760, 1720, 1660, 1530. NMR (DMSO-d_6, 60 MHz) δ: 2.90, 3.26 (2H, AB q, J = 4 Hz, C_2), 3.53 (2H, s, Ph-CH_2), 3.67—4.17 (1H, m, C_3), 4.42 (1H, d, J = 6 Hz, C_4), 5.10 (1H, d, J = 4 Hz, C_6), 5.33 (1H, dd, J = 4, 8 Hz, C_7), 7.23 (5H, s, Ph–), 9.03 (1H, d, J = 8 Hz, –CO–NH–).

7-Amino-3-hydroxycepham-4-carboxylic Acid (IX)——MsOH (383 mg) was added to a cooled suspension of X (854 mg) in dry MeOH (26 ml). The resulting clear solution was cooled to −75 °C and O_3 gas was passed through the solution until no X was detected by HPLC. After N_2 gas had been bubbled for 5 min to remove excess O_3, the solution was allowed to stand until the inner temperature rose to −50 °C, and was kept at −50 °C for additional 1 h. The solution was poured into an aqueous solution of $NaBH_4$ (prepared from $NaBH_4$ (38 mg), NaOH (400 mg) and H_2O (13 ml)) at below 10 °C. HPLC analysis showed that 722 mg (83.0%) of IX was produced. The resulting solution was

adjusted to pH 4.0 with concentrated HCl to give crystals, which were collected, washed with H_2O, MeOH and acetone, successively, and dried under reduced pressure to give IX (652 mg) in 75% yield, mp 228—236 °C (dec.). Retention time in HPLC was 3.0 min. *Anal.* Calcd for $C_7H_{10}N_2O_4S$: C, 38.53; H, 4.62; N, 12.84; S, 14.69. Found: C, 38.29; H, 4.49; N, 12.75; S, 14.91. IR ν_{max}^{Nujol} cm^{-1}: 3550, 3200–2000, 1770, 1630, 1570. NMR (D_2O–DCl, 60 MHz) δ: 3.05—3.23 (2H, m, C_2), 4.5—4.8 (1H, m, C_3), 4.85 (1H, d, $J=3$ Hz, C_4), 4.98 (1H, d, $J=3$ Hz, C_7), 5.42 (1H, d, $J=3$ Hz, C_6).

7-Amino-3-hydroxy-3-cephem-4-carboxylic Acid (XXII) Hydrochloride——O_3 gas was passed through a solution of X·HCl (6.0 g) in dry MeOH (150 ml) at −75 °C. The reaction mixture obtained was evaporated carefully under reduced pressure on a water bath at a temperature below 20 °C to give an oil, which was triturated with ether to provide a powder. The powder was collected and dried under reduced pressure to give crude XXII·HCl (6.7 g). IR ν_{max}^{Nujol} cm^{-1}: 3200—2000, 1770, 1640, 1570. NMR (CD_3OD, 100 MHz) δ: 3.72 (2H, AB q, $J=16$ Hz, C_2), 5.30 (1H, d, $J=4$ Hz, C_6), 5.50 (1H, d, $J=4$ Hz, C_7).

IX from XXII·HCl——XXII·HCl (1 g) obtained above was dissolved in dry MeOH (30 ml) and treated with $NaBH_4$ aqueous solution in a similar manner to the method used for the preparation of IX from X. Compound IX was produced in 39.9% yield from the starting X based on HPLC analysis.

XIX and 3-Methylene-7-phenylacetamidocepham-4-carboxylic Acid 1-Sulfoxide (XXIV) from X——Compound X (4.3 g) was oxidized by O_3 at −40 °C in MeOH (150 ml) following dissolution by adding MsOH (2 g), and N_2 gas was bubbled into the solution for 5 min to remove the excess ozone. The ozonized intermediate was reduced with $NaBH_4$ as stated above. The reaction mixture was adjusted to pH 7.0, and allowed to react with phenylacetyl chloride (6.3 g), the pH of the solution being maintained between 6.5 to 7.5 with aqueous Na_2CO_3 solution. The reaction mixture was concentrated under reduced pressure to remove MeOH, and the aqueous residue obtained was washed with AcOEt and then acidified with concentrated HCl to pH 2.0. The resulting solution was extracted with AcOEt (100 ml × 2). The extract was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure to give an oil (8.2 g), which was subjected to chromatography over SiO_2 (24 g) using AcOEt as an eluent. Fractions containing XXIV were collected and concentrated under reduced pressure to give crystals of XXIV (0.9 g), mp 185—191 °C. IR ν_{max}^{Nujol} cm^{-1}: 3500, 3300, 3050, 1750, 1710, 1660, 1540. NMR (DMSO-d_6, 60 MHz) δ 2.77—3.50 (3H, m, C_2 and C_3), 3.58 (2H, s, Ph–CH_2), 4.50 (1H, d, $J=6$ Hz, C_4), 5.13 (1H, d, $J=4$ Hz, C_6), 5.42 (1H, dd, $J=4$, 8 Hz, C_7), 7.37 (5H, s, Ph–), 9.07 (1H, d, $J=8$ Hz, –CO–NH–).

Fractions containing XIX were collected, and concentrated under reduced pressure to give crystals of XIX (2.1 g), the identity of which was confirmed by comparing the spectral data with those of the authentic sample.

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